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Significantly Improved Expression and Biochemical Properties of Recombinant *Serratia marcescens* Lipase as Robust Biocatalyst for Kinetic Resolution of Chiral Ester

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Abstract A lipase gene from *Serratia marcescens* ECU1010 was cloned into expression vector pET28a, sequenced, and overexpressed as an N terminus His-tag fusion protein in *Escherichia coli*. Through the optimization of culture conditions in shake flask, the lipase activity was improved up to 1.09×10^5 U/l, which is a great improvement compared to our previous reports. It was purified to homogeneity by Ni-NTA affinity chromatography with an overall yield of 59.4% and a purification factor of 2.4-fold. This recombinant lipase displayed excellent stability below 30 °C and within the pH range of 5.0−6.8, giving temperature and pH optima at 40 °C and pH 9.0, respectively. The lipase activity was found to increase in the presence of metal ions such as Ca^{2+}, Cu^{2+}, and some nonionic surfactants such as PEG series. In addition, among *p*-nitrophenyl esters of fatty acids with varied chain length, the recombinant lipase showed the maximum activity on *p*-nitrophenyl laurate (C_{12}). Using racemic *trans*-3-(4′-methoxy-phenyl)-glycidyl methyl ester [(±)-MPGM] as substrate, which is a key chiral synthon for production of diltiazem, a 50% conversion yield was achieved after 4 h in toluene–water (100 mM KPB phosphate buffer, pH 7.5) biphasic system (5:5 ml) at 30 °C under shaking condition (160 rpm), affording (−)-MPGM in nearly 100% *ee*. The K_m and V_{max} values of the lipase for (±)-MPGM were 222 mM and 1.24 $mmol\,min^{-1}\,mg^{-1}$, respectively. The above-mentioned features make the highly enantioselective lipase from *Serratia marcescens* ECU1010 a robust biocatalyst for practical use in large-scale production of diltiazem intermediate.

Keywords *Serratia marcescens* ECU1010 · Lipase · Soluble expression · Purification · Characterization · Enantioselectivity · Biocatalytic resolution

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Introduction

Lipases or triacylglycerol acyl hydrolase (EC3.1.1.3) are able to catalyze the hydrolysis of medium- and long-chain triglycerides into fatty acids and glycerol at the interface between an insoluble substrate and water and can also catalyze ester synthesis and transesterification reactions with high regioselectivity and stereoselectivity in nonaqueous medium systems. Now lipases have become an excellent biocatalyzer in a broad range of industrial application, including the food industry, biological detergents, medicinal applications, and enzymatic production of lipophilic fine chemicals [1–5]. Among various bacterial lipases, lipase from *Serratia marcescens* ECU1010 has received much attention due to its potential as useful biocatalyst for the kinetic resolution of racemic trans 3-(4′-methoxy-phenyl)-glycidyl methyl ester [(±)-MPGM], a key intermediate for the synthesis of diltiazem hydrochloride [6].

In recent years, a variety of lipase-encoding genes from different *S. marcescens* species have been cloned and sequenced, and the corresponding proteins have been expressed in homologous or heterogeneous hosts. Japanese scientists improved the enzyme production by nearly 140-fold [7] and immobilized the lipase into a hollow-fiber membrane to produce (−)-MPGM effectively [6]. Jaeger cloned the gene of *S. marcescens* SM6 lipase into a T7 expression vector (pET11d) and transformed it into *Escherichia coli* JM109 (DE3) for expression [8]. Jaeger et al. [9] have also succeeded in enzymatic resolution of racemic isopropylidene glycerol acetate (IPGA) by using the *S. marcescens* SM6 lipase.

Nevertheless, the high-level production of the active forms in heterogonous hosts like *E. coli* has not yet been achieved. The most difficult problems in bacterial expression are proteolytic degradation and the production of proteins that are accumulated in misfolded forms [10]. On the other hand, lipase is strongly hydrophobic in nature. When continuously accumulated as foreign protein in cytoplasm, lipase produces toxicity to host cells, leading to their death or forming inactive inclusion bodies [11]. Therefore, how to increase soluble lipase production in a biochemically safe and economic expression system such as *E. coli* attract a tremendous interest among molecular biologists and biochemical engineers.

In this paper, we report the cloning, fusion expression, purification, and partial characterization of the recombinant lipase from a new soil isolate, *S. marcescens* ECU1010. The recombinant lipase was highly expressed in a soluble form of protein, which means that there is a great significance in biotechnological application of the lipase for enzymatic synthesis of chiral drugs by supplying the robust *S. marcescens* lipase.

Materials and Methods

Macroorganisms and Materials

The host strain *E. coli* BL21 (DE3) and expression vector pET28a were obtained from Novagen; the strain of *S. marcescens* ECU1010 used in this work was a Gram-negative bacterium isolated in our laboratory and currently deposited at China General Microbiology Collection Center, with an accession number of CGMCC 1219. Ex-Taq DNA polymerase, T4DNA ligase, pMD-18T vector, restriction endonucleases, and DNA Marker were purchased from TaKaRa Biotechnology Co. (Dalian, China). Isopropyl-β-D-thiogalactopyranoside (IPTG) and kanamycin were purchased from Sigma (St. Louis, USA). Ni-NTA agarose was from Qiagen Co. All kinds of *p*-nitrophenyl esters were prepared from *p*-nitrophenol and various fatty acids [12]. All the other chemicals were also available commercially and of the highest reagent grade.

Cloning, Sequencing, and Overexpression of the Lipase Gene

The lipase-encoding gene was amplified from chromosomal DNA by polymerase chain reaction (PCR) using a pair of oligonucleotide primers. Forward primer was 5′-ggccatatgggc atctttagctataagg-3′ (*Nde*I cutting site is underlined); reverse primer was 5′-cctaagcttttaggccaa caccacctga-3′ (*Hin*dIII cutting site is underlined). The PCR products were then ligated into pMD18-T simple vector and sequenced by ABI 3730 DNA analyzer.

After double digestion with *Nde*I and *Hin*dIII, the *Nde*I–*Hin*dIII fragment was inserted into pET28a vector digested with the same enzymes according to the manufacturer's instructions. The recombinant plasmid was transformed into *E. coli* BL21 (DE3) for protein expression. The transformed strains were grown at 37 °C in 100 ml LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl, pH 7.0) containing kanamycin (50 μg/ml) until the OD_{600} reached 0.8–1.0. Protein expression was induced by adding IPTG and Ca^{2+} to a final concentration of 0.1 and 5 mM, respectively, and then the culture was shaken at 15 °C for 18 h, and the cells were harvested by centrifugation.

Purification of Recombinant Lipase

Ni-NTA agarose affinity chromatography was used in the purification process. Firstly, the cell pellet of *E. coli* was suspended in 20 mM phosphate buffer (pH 7.0) and centrifuged, and the supernatant was discarded. This washing process was repeated twice. Secondly, the washed cells of *E. coli* were suspended in 10 ml cold binding buffer (50 mM Tris–HCl/ 100 mM NaCl, pH 8.0) and lysed by sonicating at 400 W using a 50% pulsed mode for 15 min. After centrifugation at 12,000 rpm (10,000×*g*) for 10 min, the supernatant was used for purification. The column (ø1.0 cm×100 cm) was equilibrated with 50 mM Tris–HCl (pH 8.0) containing 0.1 M NaCl at a flow rate of 30 ml/h, and the sample was loaded with the same buffer, and then it was eluted with the 50 mM NaAc-HAc buffer (pH 5.0). The active fractions were collected, and their activities were measured. The purity of lipase was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Assay of Lipase Activity and Protein Concentration

Lipase was assayed using *p*-nitrophenyl acetate (*p*NPA) as the substrate. Lipase or blank solution (100 μl) was added to 2.870 ml of 100 mM potassium phosphate buffer (KPB, pH 7.5). After preincubation at 30 °C for 3 min, the reaction was initiated by a quick mixing of the reaction mixture with 30 μl of 100 mM *p*NPA solution in dimethyl sulfoxide (DMSO), and the variation in absorbance at 405 nm was recorded. One unit of lipase activity was defined as the amount of enzyme releasing 1.0 μmol of *p*-nitrophenol per minute under such conditions. Hydrolytic activities on various *p*-nitrophenyl esters were measured by a modified lipase assay method. *p*-Nitrophenyl butyrate was replaced with various *p*-nitrophenyl esters, respectively. Particularly, for *p*-nitrophenyl laurate, myristate, and palmitate, lipase or blank solution (100 μl) was added to 2.57 ml of 100 mM potassium phosphate buffer (KPB, pH 7.5). After preincubation at 30 °C for 3 min, the reaction was initiated by a quick mixing of the reaction mixture with 300 μl of 10 mM *p*-nitrophenyl laurate, myristate, or palmitate solution in DMSO, and the variation in absorbance at 405 nm was recorded. All the assays were performed in triplicate, and the average values were taken.

The protein concentration was assayed using Bradford method with bovine serum albumin as the standard [13].

Enzyme Characterization

The effect of temperature on the hydrolytic activity was determined by measuring the hydrolytic activity at different temperatures ranging from 25 to 65 °C. The optimum pH was determined by measuring the hydrolytic activity in buffers with various pH values.

The stability of the lipase to temperature was investigated by measuring the residual activity after incubating the purified lipase at 30, 40, and 50 °C for 0.5–2 h. The effect of pH on lipase stability was determined by assaying the residual activity after incubating the purified lipase in the buffers with different pH values at 4 °C for 20 h.

The effects of ethylenediaminetetraacetic acid (EDTA), various metal ions, and surfactants on the hydrolytic activity were determined by measuring the residual activity after incubating the purified lipase containing EDTA (2 mM), each of various metal ions (2 mM), and each of surfactants (0.1%, *w*/*v*) in a 50 mM Tris–HCl buffer (pH 8.0) at 30 °C for 0.5 h, respectively.

The substrate specificity of the lipase was studied using *p*-nitrophenyl fatty acid esters with various acyl chain lengths under standard conditions.

The Michaelis–Menten kinetic parameters (V_{max} and K_m) of the lipase were calculated using (±)-MPGM as the substrate (substrate concentration 10, 25, 50, 100, 200 mM, respectively). Lineweaver–Burk plots were used to calculate V_{max} and K_m parameters, assuming that the reaction follows a simple Michaelis–Menten kinetics.

Enantioselective Hydrolysis

The hydrolysis of *trans*-3-(4-methoxy-phenyl)glycidic acid methyl ester, (±)-MPGM, was carried out in toluene–water (100 mM KPB phosphate buffer, pH 7.5) biphasic system (5:5 ml). After the purified lipase was added, the mixture was incubated at 30 °C with shaking at 160 rpm. Samples of the toluene layer were dried over anhydrous sodium sulfate for high-performance liquid chromatography (HPLC) analysis. The conversion (*c*) and enantiomeric excess (*ee*) were determined by means of HPLC according to previous reports [14–21].

Results and Discussion

Cloning and Gene Analysis

A pair of oligonucleotide primers was designed based on the nucleotide sequences immediately upstream and downstream of the known coding sequences of lipase. Using these primers, a band of about 1,845-bp length was amplified from the chromosomal DNA of *S. marcescens* ECU1010. Sequencing and gene analysis indicated a full-length lipase gene motif encoding a polypeptide of 614 aa residues, which shares 94–96% identities on DNA level and 96–98% identities on amino acid level with those of *S. marcescens* Sr41, *S. marcescens* SM6, and *S. marcescens* ES-2, respectively [22, 23]. Sequence alignment revealed that the active site of the lipase contains a catalytic triad consisting of Ser–His–Asp/Glu, but unlike most serine proteases, the active site is buried inside the structure. A “lid” or “flap” covers the active site, making it inaccessible to solvent and substrates. The lid opens during the process of interfacial activation, allowing the lipid substrate access to the active site.

Expression of the Recombinant Protein

The *lip* gene was then overexpressed in *E. coli* because many physical and chemical factors such as inducers, incubation time, and temperature may affect the production and correct folding of lipase overexpressed in *E. coli*. For the recombinant strain, we firstly optimized the induction conditions to enhance the soluble expression of the lipase gene. After optimization, the lipase production was enhanced to 2.14×10^4 U/l when induced by adding IPTG to a final concentration of 0.1 mM at 15 °C for 18 h, which represents a significant improvement to our previous reports [14, 24, 25]. From the SDS-PAGE (Fig. 1), it can be seen that the soluble fraction of recombinant lipase expressed at 15 °C has been greatly enhanced as compared to that induced at 37 °C. The lipase expressed in soluble fraction was estimated to be about 26% of the total proteins in the cell-free extract after optimization. Figure 1 reveals the presence of a single protein with a molecular weight of 65 kDa, which is a relatively high value among other known lipases from bacteria [26].

Interestingly, it was found that the activity of the protein was significantly improved by the addition of Ca^{2+} during IPTG induction. Adding Ca^{2+} to a final concentration of 5 mM increased the activity of lipase by nearly fivefold with respect to the control (2.14×10^4 U/l), reaching 1.09×10^5 U/l. This suggests that the Ca^{2+} binding may involve the active site of the lipase.

Effect of Different Fusion Tags on Lipase Expression

It is worth stressing here, under the same experimental conditions, that the bacterial density of each control group was almost the same, but the activity of the pET28a lipase was much higher than the other recombinant lipases we had constructed (Table 1). It might be due to the difference in protein expression; on the other hand, we are more inclined to conclude that the size of fusion tag has an effect on the activity and solubility of the recombinant protein (related studies are still in progress). The existence of fusion tags such as GST, Trx,

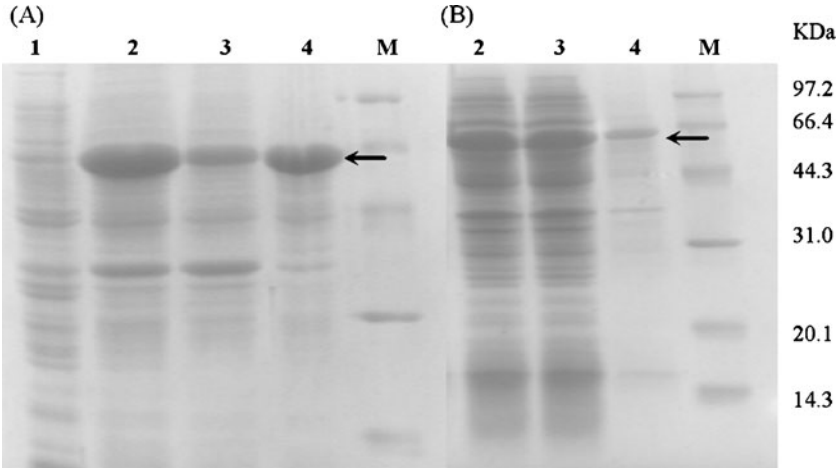

Fig. 1 SDS-PAGE analysis. (*A*) 37 °C for 4 h before optimization; (*B*) 15 °C for 18 h under optimized conditions. *Lane 1*: uninduced cell sample; *lane 2*: induced cell sample; *lane 3*: supernatant of cell lysate; *lane 4*: pellet of cell lysate; *lane M*, molecular weight marker. The gel was stained by Coomassie brilliant blue R-250. The *arrow* indicates the position of the fusion protein (65 kDa)

Table 1 Comparison of lipase expression on vectors with different fusion tags.

Strain/plasmid	N terminus fusion tag	Size of fusion tag (aa)	Activity (U/l)	Reference
S. marcescens ECU1010	–	–	4,780	[14]
pET24a	None	–	5,000~6,000	[25]
pET28a	His-tag	6	21,400	This study
pET32a	Trx-Tag, His-tag, S-Tag	130	2,750	Unpublished data
pGEX-4T-1	GST	231	1,980	Unpublished data

All of the experimental group above were not added with Ca^{2+}

etc. is in favor of soluble expression, but macromolecular fusion tags may contribute to the incorrect folding of spatial structure of fusion protein, then resulting in either misfolding or instability, which can be reflected by comparing pGEX-4T-1 lipase and pET32a lipase with pET28a lipase.

Purification of the Recombinant Protein

We have reported the purification of native lipase from *S. marcescens* ECU1010, by ultrafiltration, $(NH_4)_2SO_4$ fractionation, DEAE-Toyopearl, Sephadex G-150 and Phenyl-Toyopearl, achieving 5.5-fold purification and 15.8% yield [27]. In this study, we constructed the N-terminally His_6-tagged fusion protein to facilitate the purification of lipase by Ni-NTA agarose affinity chromatography, using standard procedures, as described in "Materials and Methods." After a series of purification procedures, the lipase was purified 2.4-fold with an overall yield of 59.4%. Figure 2 shows the SDS-PAGE of the effluent with lipolytic activity, indicating a single protein band of lipase. Thus, the single-step purification of recombinant *S. marcescens* ECU1010 lipase is a simple and effective method using metal-affinity chromatography (Table 2).

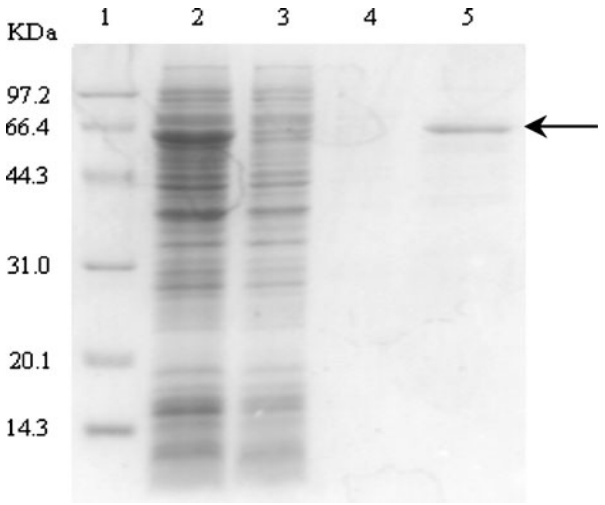

Fig. 2 Purification of recombinant *S. marcescens* ECU1010 lipase. *Lane 1*, molecular mass marker proteins; *lane 2*, supernatant of cell-free extract; *lane 3*, flow through; *lane 4*, wash; *lane 5*, eluates with 50 mM NaAc-HAc buffer (pH 5.0). The *arrow* indicates the position of the fusion protein (65 kDa)

Table 2 Summary of the recombinant lipase purification.

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
Supernatant of cell lysate	10.4	24.9	2.4	1	100
Ni-NTA Agarose	2.6	14.8	5.7	2.4	59.4

The activity was assayed using *p*-nitrophenyl acetate as substrate

Effects of Temperature and pH on Activity and Stability

The temperature–activity curve (Fig. 3a) shows that the optimal reaction temperature is 40 °C. The purified lipase keeps not less than 80% of the maximum activity at 40 °C in a temperature range of 25−45 °C. After being incubated at temperatures of 30, 40, and 50 °C for 0.5−2 h, the enzyme has a remaining residual activity of over 90% at 30 °C compared with the initial activity before incubation. However, only 0.5 h later, the residual activity decreases sharply when the temperature is above 50 °C (Fig. 3b). The purified lipase shows an optimum hydrolytic activity at pH 9.0 (Fig. 4a), and this is distinguished from most lipases whose optimum pH are in the range of pH 7.0–8.0 [5]. It exhibits no less than 80% of the maximum activity at pH over 8.2, while no activity is detected at pH below 6.0. Furthermore, the pH stability curve (Fig. 4b) of the lipase shows that the enzyme is stable over a pH range of 5.0–6.8 with more than 80% residual activity after being incubated at 4 °C for 20 h.

Effects of Metal Ions and Surfactants on Lipase

Metal cations, particularly Ca^{2+}, play important roles in influencing the structure and function of enzymes, and many calcium-stimulated lipases have been reported [28]. The recombinant lipase is no exception, since Table 3 indicates that 2 mM Ca^{2+} increases the activity by 100% with respect to the blank control, whereas other metal ions like Fe^{2+}, Cu^{2+}, etc. can maintain or enhance the lipase activity. On the contrary, Zn^{2+} inhibits nearly 20% activity; the chelating agent EDTA significantly inhibits the activity of the lipase by 75%. These properties possibly suggest that the lipase may be a metalloenzyme; Ca^{2+} is essential for its

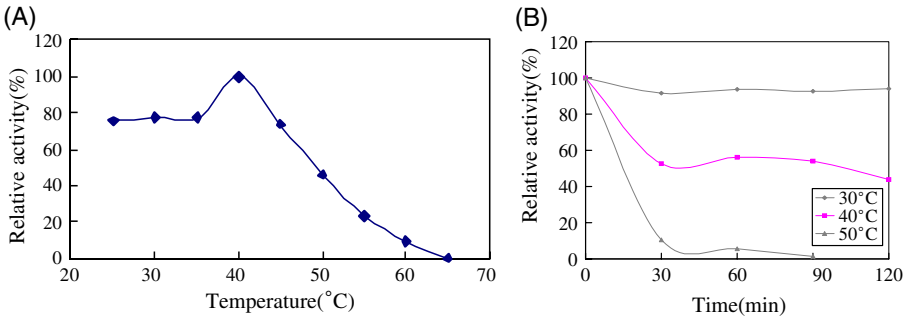

Fig. 3 Effects of temperature on activity (**a**) and stability (**b**) of the lipase from *S. marcescens* ECU1010. **a** Temperature–activity was determined by measuring the relative activity at different temperatures. **b** Temperature–stability was determined by measuring the residual activity after incubating the purified lipase at 30, 40, and 50 °C for 0.5–2 h

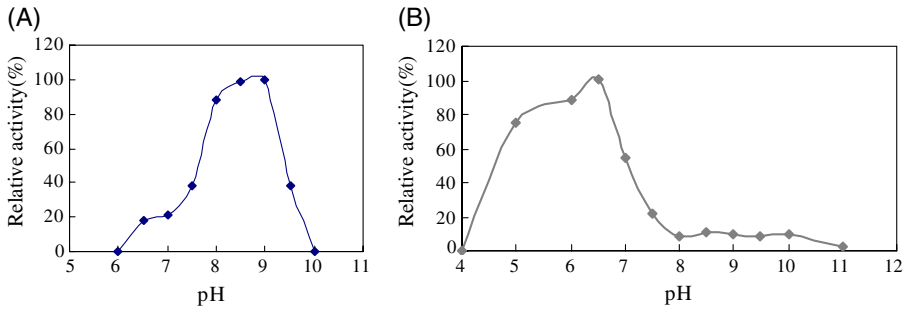

Fig. 4 Effects of pH on activity (**a**) and stability (**b**) of the lipase from *S. marcescens* ECU1010. **a** Purified lipase samples were assayed in various buffer from pH 6.0 to pH 10.0 using *p*NPA (100 mM) as substrate at 40 °C. **b** Purified lipase samples were diluted in buffer with different pH values, pH-adjusted, and incubated for 20 h at 4 °C

activity [29]. However, this result is somewhat different from native lipase from *S. marcescens* ECU1010 which was reported as not significantly enhanced by Ca^{2+} [27].

Surfactants have been widely applied to lipase-catalyzed reactions of insoluble substrates to increase the lipid–water interfacial area, which in turn enhances the enantioselectivity as well as the reaction rate of the kinetic resolution [30]. In addition, surfactants are also involved in hydrogen bonds and hydrophobic action between lipases and surfactants, which can change the enantioselectivity of the lipase toward (*S*)-ketoprofen ester [31, 32].

As shown in Table 3, nonionic surfactants PEG series and PVA at 0.1% (*v*/*v*) increase the activity of the lipase by 1.8~16%, while Triton X-100 and Tween-20 at 0.1% (*v*/*v*) reduce its activity by 98% and 20%, respectively. Anionic surfactant SDS almost inhibits the

Table 3 Effects of various metal ions, EDTA, and various surfactants on the activity of recombinant *S. marcescens* lipase.

Metal ions/surfactants	Concentration (mM)	Relative activity (%)
Blank	–	100.0
Ca^{2+}	2	200.2
Fe^{2+}	2	100.3
Mn^{2+}	2	100.8
Ni^{2+}	2	172.5
Cu^{2+}	2	172.5
Mg^{2+}	2	106.5
Zn^{2+}	2	80.0
Ba^{2+}	2	130
Li^{+}	2	140
EDTA	2	25.3
Tween 20	0.1% (*w*/*v*)	80.1
Triton X-100	0.1% (*w*/*v*)	2.3
PVA	0.1% (*w*/*v*)	111.4
PEG1500	0.1% (*w*/*v*)	101.8
PEG4000	0.1% (*w*/*v*)	116.0
PEG20000	0.1% (*w*/*v*)	105.9
SDS	0.1% (*w*/*v*)	20.1

S. marcescens ECU1010 lipase was incubated with various metal ions and surfactants at 30 °C for 30 min before the activity was measured with *p*-nitrophenyl acetate. All assays were performed in duplicates. The activity toward *p*-nitrophenyl acetate without anything was taken as 100%

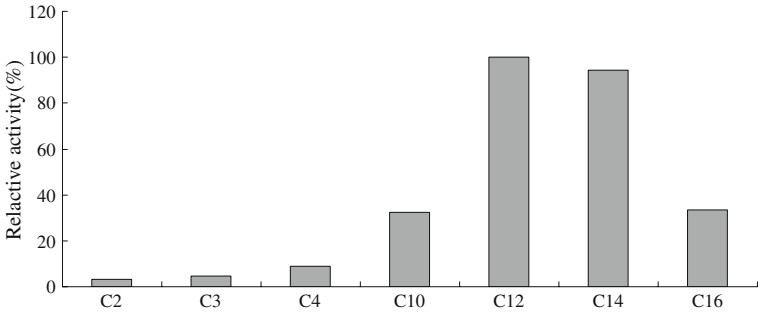

Fig. 5 Substrate specificity of the recombinant *S. marcescens* lipase towards *p*-nitrophenyl fatty acid esters with various acyl chain lengths

hydrolytic activity of the lipase by 80%. Based on the results above, we can optimize the activity by selectively adding metal ions and/or proper surfactants to the reaction system.

Substrate Specificity of Lipase

Substrate specificity of the lipase was investigated with various *p*-nitrophenyl esters. The esters of various fatty acids with a moderate to long chain (N_C>10) served as good substrates for the enzyme, and *p*-nitrophenyl laurate (C_{12}) was the best among all the substrates tested, as shown in Fig. 5. Therefore, the enzyme is a typical lipase which prefers a long-chain lipid, though it also displayed a strong carboxylic esterase activity as well. Whereas in the literature [26, 32] a lipase from *S. marcescens* Sr41 8000 showed the highest activity toward triacylglycerides of relatively shorter chains (C_4–C_8), another lipase from *S. marcescens* ES-2 preferably hydrolyzed the triacylglycerides of medium chains (C_8–C_{12}).

Kinetic Parameters

The kinetic parameters for *S. marcescens* ECU1010 lipase were determined by Lineweaver–Burk plot (Fig. 6). It showed a K_m of 222 mM and V_{max} of 1.24 mmol$min^{-1}$$mg^{-1}$ towards (±)-MPGM as substrate. These are similar to those reported for the lipase from another strain of *S. marcescens* Sr41 8000 [33].

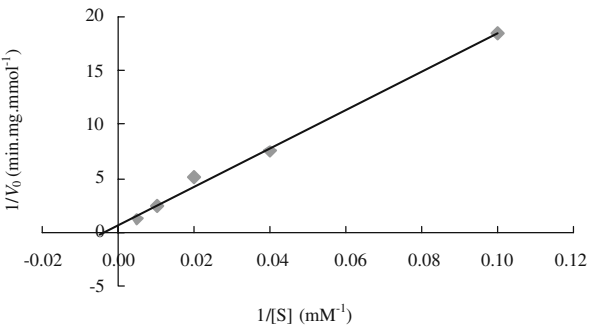

Fig. 6 Lineweaver–Burk plot for lipase. Recombinant *S. marcescens* lipase was assayed using varying concentrations of (±)-MPGM. Lineweaver–Burk plot was used to calculate the kinetic parameters, K_m and V_{max}

Table 4 Enantioselective hydrolysis of a chiral ester, (±)-MPGM, by *S. marcescens* lipase.

Substrate	Concn. (mM)	Time (h)	Conv. (%)	ee_s (%)	Enantioselectivity (*E*-value)
(±)-MPGM	100	2	43.8	78	>100
(±)-MPGM	100	4	50.0	100	>100

S. marcescens ECU1010 lipase was incubated in toluene–water biphasic system at 30 °C under shaking condition (160 rpm) before the lipase activity was measured by the *p*-nitrophenyl acetate (*p*NPA) substrate. All assays were performed in duplicates

Potential Application of the Lipase for Kinetic Resolution of Chiral Esters

(±)-MPGM was chosen as a typical example for kinetic resolution of chiral carboxylic acid esters. The result was shown in Table 4. As shown in Fig. 7a, the reaction proceeded with 43% conversion at a substrate concentration of 1.0 M after 2 h of reaction; and at 4 h (Fig. 7b), the conversion had reached 50% with 100% enantiomeric excess (*ee*), resulting in a highly pure product, *trans*-(2*R*, 3S)-(−)-MPGM, which is known as a key intermediate for

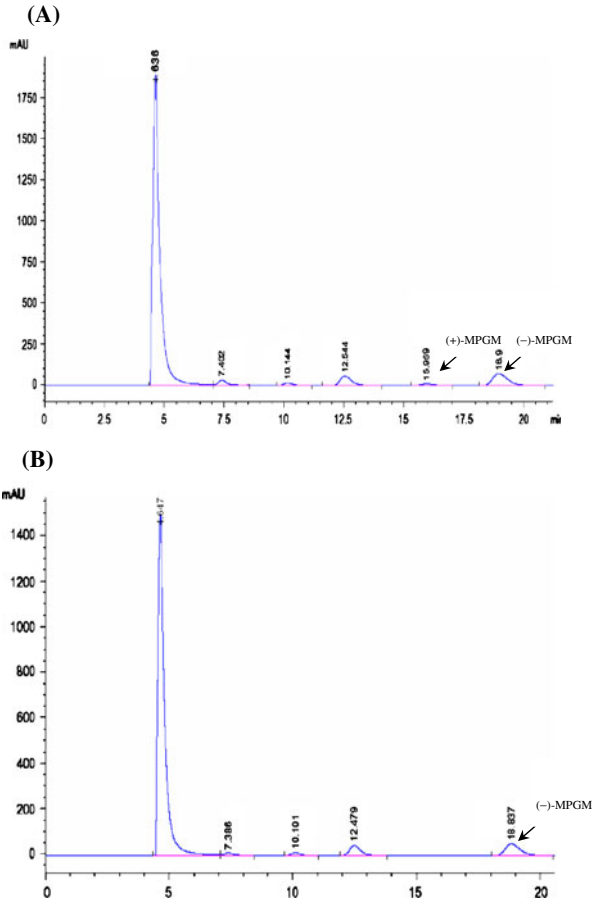

Fig. 7 HPLC analysis of the biohydrolysis reaction mixture with (±)-MPGM as substrate: **a** 2 h later, **b** 4 h later. Peaks corresponding to (+)-MPGM and to (−)-MPGM are indicated by *arrows*

production of an important antihypertensive agent diltiazem. Besides, this lipase and other lipases from *S. marcescens* SM6 and *S. marcescens* ES-2 had also been used for kinetic resolution of other racemic mixtures, including menthyl acetate, glycidyl butyrate, isopropylidene glycerol acetate, 2-phenyl-1-propanol, and (*S*)-flurbiprofen [8, 26, 27]. All those suggest that further improvement of the reactivity and selectivity of *S. marcescens* lipase may be expected in the field of kinetic resolution.

Conclusions

In this study, we have successfully cloned a lipase gene from *S. marcescens* ECU1010 and expressed it at the high-level production of 1.09×10^5 U/l lipase in the supernatant under optimum conditions by adding Ca^{2+}. Meanwhile, through the comparison of related experiments, we found that small molecular weight fusion tag was more conducive to soluble expression of the active lipase, which might be due to the incorrect folding of fusion protein caused by macromolecule fusion tag.

This recombinant lipase displayed good stability below 30 °C and at pH 5.0–6.8, with the temperature and pH optima at 40 °C and pH 9.0, respectively. Different from native lipase, it was activated by Ca^{2+} significantly and showed the maximum activity on *p*-nitrophenyl laurate (C_{12}) among various *p*-nitrophenyl esters. In addition, the most noteworthy of the lipase is its remarkable enantioselectivity to (±)-MPGM in organic solvents. All these results provide sufficient advantages to make *S. marcescens* ECU1010 lipase a promising candidate for the biocatalytic preparation of diltiazem. Meanwhile, this work will be of great value to both the efficient expression in *E. coli* as a host cell and the large-scale production of *S. marcescens* ECU1010 lipase which is being or will be widely used in organic synthesis.

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